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Biomarkers in the diagnosis of pleural diseases: a 2018 update

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***Abstract*:** The use of biomarkers on pleural fluid (PF) specimens may assist the decision-making process and enhance clinical diagnostic pathways. Three paradigmatic examples are heart failure, tuberculosis and, particularly, malignancy. An elevated PF concentration of the amino-terminal fragment of probrain natriuretic peptide (>1500 pg/ml) is a hallmark of acute decompensated heart failure. Adenosine deaminase, interferon-γ and interleukin-27 are three valuable biomarkers for diagnosing tuberculous pleurisy, yet only the first has been firmly established in clinical practice. Diagnostic PF biomarkers for malignancy can be classified as soluble-protein based, immunocytochemical and nucleic-acid based. Soluble markers (e.g. carcinoembryonic antigen (CEA), carbohydrate antigen 15–3, mesothelin) are only indicative of cancer, but not confirmatory. Immunocytochemical studies on PF cell blocks allow: (a) to distinguish mesothelioma from reactive mesothelial proliferations (e.g. loss of BAP1 nuclear expression, complemented by the demonstration of p16 deletion using fluorescence *in situ* hybridization, indicate mesothelioma); (b) to separate mesothelioma from adenocarcinoma (e.g. calretinin, CK 5/6, WT-1 and D2-40 are markers of mesothelioma, whereas CEA, EPCAM, TTF-1, napsin A, and claudin 4 are markers of carcinoma); and (c) to reveal tumor origin in pleural metastases of an unknown primary site (e.g. TTF-1 and napsin A for lung adenocarcinoma, p40 for squamous lung cancer, GATA3 and mammaglobin for breast cancer, or synaptophysin and chromogranin A for neuroendocrine tumors). Finally, PF may provide an adequate sample for analysis of molecular markers to guide patients with non-small cell lung cancer to appropriate targeted therapies. Molecular testing must include, at least, mutations of epidermal growth-factor receptor and BRAF V600E, translocations of rat osteosarcoma and anaplastic lymphoma kinase, and expression of programmed death ligand 1.

Keywords: adenosine deaminase, BAP1, biomarkers, carcinoembryonic antigen, epidermal growth-factor receptor, interleukin-27, mesothelin, natriuretic peptides, pleural effusion

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Introduction

Pleural effusion (PE) is a common clinical problem.[1] Heart failure (HF), malignancy, pneumonia and tuberculosis (TB) account for three quarters of all cases.[2] The search for the cause of PE almost always involves the analysis of pleural fluid (PF) obtained by thoracentesis. Unfortunately, many PF analyses lack enough sensitivity or specificity, which may ultimately lead to requesting invasive procedures such as closed pleural biopsies and thoracoscopies.[3] The measurement of a number of potentially accurate biomarkers on PF specimens for the identification of HF, TB and cancer has simplified clinical diagnostic pathways.[4] The current review briefly summarizes the main molecules that can be used in current practice for a convenient and non-invasive approach to PE, with a particular emphasis on malignant etiology.

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Biomarkers of heart-failure-related effusions

The incidence of PEs in patients with acute decompensated HF is high. In a recent retrospective review of 3245 consecutive patients with HF from a Spanish registry, PEs were observed on

chest radiographs in 46% of the cases, and their distribution was bilateral in 58%, isolated right sided in 27% and isolated left sided in 14%.[5] If the method of detection is ultrasonography or computed tomography (CT) rather than simple radiographs, the frequency of HF-related effusions greatly increases.

The diagnosis of PEs due to HF is not always obvious. One study showed that clinical judgment prior to thoracentesis failed in the identification of transudates more than 40% of the time,[6] while the standard Light's criteria were more informative, though they still misclassify 25–30% of cardiac effusions as 'exudates'.[7,8] The correct identification of these 'false cardiac exudates' has attracted the interest of clinicians, who can adopt different strategies to circumvent the problem, the most straightforward being the use of cardiac-specific biomarkers.[9]

Natriuretic peptides, specifically BNP and amino-terminal fragment of probrain natriuretic peptide (NT-proBNP), are now established biomarkers for supporting the diagnosis of HF. A meta-analysis of 12 studies, totaling 599 cardiac and 1055 noncardiac effusions, showed that PF NT-proBNP (usually at a threshold of $\geqslant$1500 pg/ml) had 94% sensitivity, 91% specificity, likelihood ratio (LR) positive of 10.9, LR negative of 0.07 and area under the curve (AUC) of 0.96 for labeling HF-related effusions.[10] In PF, NT-proBNP is a more accurate biomarker than BNP.[11] Because of the strong correlation between PF and serum levels, blood measurements may suffice in many circumstances. However, if thoracentesis is already planned in order to rule out noncardiac causes or mixed conditions, determination of NT-proBNP in PF rather than serum is the preferred method. Notably, about 80% of misclassified cardiac effusions using Light's criteria have PF NT-proBNP levels $>$ 1500 pg/ml.[9]

When natriuretic peptide tests are not available, the reclassification of miscategorized cardiac effusions is commonly done by calculating either the serum-to-PF-albumin gradient (serum albumin minus PF albumin) or the serum-to-PF-protein gradient. As in the case of natriuretic peptides, around 80% of 'false cardiac exudates' exhibit an albumin gradient $>$ 1.2 g/dl or a protein gradient $>$ 2.5 g/dl.[12] However, in contrast to the former, the latter two are far from being specific, since 30% or more of noncardiac exudates also surpass these cutoffs.[12] In fact, the albumin and protein gradients only slightly change the probability that an exudate is of cardiac origin (respective LRs positive of 2.4 and 2.7, and LRs negative of 0.33 and 0.30).[12] Recently, a scoring system has been devised and validated to assist clinicians in estimating the probability of HF in patients whose PFs meet Light's exudative criteria.[12] The score consisted of age $\geqslant$ 75 years (3 points), albumin gradient $>$ 1.2 g/dl (3 points), bilateral effusions on chest radiograph (2 points), PF lactate dehydrogenase $<$ 250 U/l (2 points) and protein gradient $>$ 2.5 g/dl (1 point). At the best cutoff of $\geqslant$7 points, the score yielded 63% sensitivity, 95% specificity, LR positive of 12.7, and LR negative of 0.39 for labeling cardiac effusions among exudates. Based on current evidence, determination of NT-proBNP in PF (or alternatively serum) should be the favored test whenever HF is a consideration in an undiagnosed PE.

Biomarkers of pleural tuberculosis

Pleural TB has traditionally been considered a paucibacillary disease. The results of 14 studies,[13–26] comprising more than 4800 patients with tuberculous PEs, give the following approximate mean sensitivities for the microbiological studies: acid-fast smears (Ziehl-Neelsen or auramine) of sputum 11%,[13,14,18,21–23,25] cultures of sputum on solid (Lowenstein-Jensen or Ogawa) or liquid media 42%,[13–15,18,21–23,25] acid-fast smears of PF 4%,[13,17,18,20–23,25,26] cultures of PF on solid or liquid media[13–22,24–26] 31%. The higher superiority claims of liquid culture media reported in two studies which summed up 440 TB pleuritis[15,16] (63% of positive cultures on PF[15,16] and 55% on sputum samples[15]) contrast with a later study of 354 patients with TB pleurisy in which the sensitivity of a BACTEC MGIT culture method on PF was just 18%.[26] Even so, the current recommendation is to culture PF and sputum specimens using both solid and liquid culture media. The low yield of cultures and the time they take to become positive (4–6 weeks for solid media and 2 weeks for liquid media) preclude immediate clinical decisions and timely treatment. The use of nucleic-acid amplification tests, such as Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA), is also suboptimal for the detection of *Mycobacterium tuberculosis* in PF. A meta-analysis of 24 studies including 2486 patients reported the pooled sensitivity of GeneXpert® (Cepheid, Sunnyvale, CA, USA) as 51.4% and 22.7%, using PF culture and a composite reference standard as the benchmark, respectively.[27] It should be stressed that molecular assays require a relatively high number of bacilli to achieve adequate sensitivity.

The limitations of bacteriological tests have stimulated the search for TB fluid biomarkers, among which the most widely supported is adenosine deaminase (ADA), a predominant T-lymphocyte enzyme. A recent meta-analysis of 16 Spanish studies, totaling 4147 patients, showed that PF ADA had 93% sensitivity, 92% specificity, LR positive of 12, LR negative of 0.08, and AUC of 0.968 for identifying TB effusions.[28] The high diagnostic accuracy of ADA was independent of the technique used for its measurement, whether colorimetric Giusti, manual or automated kinetic methods. The commonly accepted ADA diagnostic cutoff is >35 U/l. ADA may be falsely low in elderly patients and falsely elevated in bacterial pleural infections (particularly complicated parapneumonic effusions and empyemas) and lymphomas.[29] Measurement of ADA2, the predominant isoenzyme in TB effusions,[30] increases specificity over total ADA in diagnosing TB pleurisy. However, its use has not been widespread because currently available ADA2 assays are not standardized and, overall, the test adds little to total ADA in the majority of cases.[31]

Only two biomarkers have equaled the accuracy of PF ADA for TB effusions: unstimulated interferon-γ and interleukin-27 (IL-27), both measured by enzyme-linked immunosorbent assays. The presence of free, unstimulated interferon-γ in PF had a respective sensitivity and specificity of 89% and 97%, with an AUC of 0.99, in a meta-analysis of 22 studies which included 2883 patients.[32] Test limitations include high cost and lack of broadly accepted discriminative cutoffs. Conversely, interferon-γ release assays (IGRAs), which quantify interferon-γ released by T lymphocytes in response to stimulation by specific mycobacterial antigens, are of little value for diagnostic purposes. Thus, in a meta-analysis of 14 studies involving 932 patients with TB effusions, PF IGRAs (either T-SPOT.TB (Oxford Immunotec, Oxford, UK) or QuantiFERON (Qiagen, Hilden, Germany) assays) displayed a pooled sensitivity and specificity of just 72% and 78%, respectively.[33]

IL-27, a member of the interleukin-12 family, mediates interferon-γ production and T-helper type 1 responses. To date, three meta-analyses have reported the diagnostic usefulness of PF IL-27 for TB effusions.[34–36] The most comprehensive one summarized seven studies (mostly from China) with a total of 1157 patients with PEs.[35] The main pooled estimates were as follows: sensitivity 93.8%, specificity 91.7%, LR positive 29.8, LR negative 0.08, and AUC 0.976. Similarly to interferon-γ, optimal cutoff values for IL-27 need to be determined. Assay technology as well as experience favor the simple and inexpensive ADA test.

Regardless of the prevalence of TB in a specific geographical area, the presence of low PF levels of ADA, interferon-γ or IL-27 implies that the post-test probability of TB is sufficiently low to reliably rule out the disease. Conversely, in high TB prevalence settings, any of these three biomarkers could be used as a rule-in test for TB effusions when they surpass threshold values, thus avoiding the need for invasive pleural biopsies.[37] In our center, located in a geographical area with a moderate incidence of TB (14.4/100,000 inhabitants in 2015) and low prevalence of multidrug-resistant TB (1.4%), closed pleural biopsies are performed in less than 10% of the suspected cases; PF ADA being the cornerstone of the diagnosis.[37]

Biomarkers of malignant effusions

The application of cancer biomarkers in PF is amply justified because: (a) cytological studies of PF are insensitive; (b) a precise cytomorphological diagnosis may be challenging; and (c) phenotyping malignant PEs is paramount in the era of personalized medicine. Consequently, a number of soluble-protein based, immunocytochemical (ICC) and molecular (mainly nucleic-acid based) markers for each of these, respectively, are now available for clinical use in order to improve categorization and management of malignant PEs.

Conventional PF cytology is negative in up to 40% of malignant PEs, and in an even greater proportion of mesotheliomas and squamous-cell lung carcinomas (~70–85%)[2,38] Cytological yield is influenced not only by tumor type, but also the cytologist's experience, number of specimens submitted for analysis, the way in which the specimens are examined and, possibly, the volume of PF processed.[3] The optimal amount of PF for cytologic examination is uncertain, but probably 20–40 ml is enough.[39] The submission of more than two separate specimens does not increase sensitivity sufficiently to be clinically meaningful.[40] Notably, standard cytological techniques should include the preparation of smears (Papanicolaou or May-Grünwald-Giemsa stainings) and cell blocks (CBs) (hematoxylin and eosin staining), since both are complementary. Thus, a recent study of 414

patients with malignant PEs demonstrated that 11% of PF samples found to be negative by cytologic smears showed malignant cells on CBs, whereas 15% of negative CBs were reported as positive on smear slides.[40]

The need for a confirmatory pleural biopsy, *via* thoracoscopy or CT guidance, in patients with suspected malignant PEs and negative PF cytological studies varies widely. It depends on whether an antineoplastic treatment is planned or only palliation, the existence of other metastatic locations that makes the demonstration of pleural invasion superfluous, the tumor type (e.g. a histological examination is desirable in mesothelioma), or local practices.[37]

Soluble-protein biomarkers

Many soluble molecules (i.e. those that can be measured in PF supernatants) have long been tested as surrogate markers of pleural metastases. A paradigmatic example is the measurement of classical tumor markers in PF, particularly CEA and carbohydrate antigen 15–3 (CA 15–3),[41] either in isolation or combination.[42,43] Overall, studies that evaluate tumor markers in patients with undiagnosed effusions have several shortcomings. First, they generally propose cutoff points with high, but not absolute specificity. And, for tumor markers to be diagnostically useful, they must be 100% specific (i.e. threshold levels should not be exceeded by any benign effusion), even though this makes tests more insensitive.[41,44] Second, to calculate cutoffs and operating characteristics, most studies include benign effusions which are easily differentiated from malignancies (e.g. empyemas or cardiac effusions). Finally, PF tumor markers are only of interest in the subset of patients with suspicious malignant PEs and negative cytological results, since for those with a positive PF cytology the diagnosis of malignancy has already been established. A recent study avoided these problems by evaluating the diagnostic accuracy of PF concentrations of CEA and CA 15–3 in 1575 patients with nonpurulent exudates.[44] Using threshold values with 100% specificity, it was found that 41%, 40% and 60% of malignant PE patients had PF levels of CEA $>$ 45 ng/ml, CA 15–3 $>$ 77 IU/l, or either, respectively. Taken together, the pooled sensitivities of these markers in combination were very similar to those of PF cytology. More importantly, more than one third of cytology-negative malignant PEs could be identified by at least one marker. However, the use of tumor markers does not prevent obtaining a cytohistological confirmation of malignancy in patients who are candidates for receiving an active oncologic treatment.

Mesothelioma is traditionally difficult to diagnose due to the low sensitivity of a PF cytological examination, usually quoted at around 30%.[2–4] Unfortunately, there are no soluble biomarkers with acceptable accuracy to identify this primary pleural tumor. Investigational biomarkers include mesothelin, osteopontin and fibulin-3, of which only the first has received US Food and Drug Administration approval for clinical use.[45] Soluble mesothelin is more sensitive when measured in PF than in serum (79% and 61%, with pooled estimates of specificity of 85% and 87%, respectively, in one meta-analysis).[46] According to the recently published British Thoracic Society guidelines, mesothelin is not recommended in isolation as a diagnostic test, but may have a place in patients with suspicious cytology who are unsuitable for or decline more invasive diagnostic procedures, provided the pretest probability of mesothelioma is high.[47] Moreover, meta-analyses of serum osteopontin (sensitivity 57%, specificity 81%)[48], blood fibulin-3 (sensitivity 62%, specificity 82%)[49] and PF fibulin-3 (sensitivity 73%, specificity 80%)[50] have shown little merit in offering useful diagnostic information for mesothelioma.

Immunocytochemical markers

CBs are usually the substrate upon which ICC and molecular studies are performed. ICC markers are commonly employed in three challenging scenarios:[4] (a) separating benign from malignant mesothelial proliferations; (b) distinguishing mesothelioma from adenocarcinoma (or other tumors); and (c) discovering the primary tumor origin of a malignant PE. The two former situations raise the question of whether a definitive diagnosis of mesothelioma can be established by PF cytologic examination alone. It is well known that cytology is not a sufficiently sensitive diagnostic test for mesothelioma, with reported figures of 16–73%, which are dependent on the cytologist's experience.[47] Moreover, two caveats of PF cytology should be considered:[51] one is that malignant cells in sarcomatoid mesothelioma are not usually shed into effusion fluid and, therefore, cytological diagnosis is limited to the epithelioid subtype; the

second is the inability of exfoliative cytology to demonstrate tissue invasion into the lung or chest wall, which is a key feature in the pathological diagnosis of mesothelioma. Recently published British guidelines state that PF cytology alone should not be relied on to make a diagnosis of mesothelioma unless pleural biopsy (*via* image guidance or thoracoscopy) is not possible or not required to determine treatment.[47]

Benign versus *malignant mesothelial cell proliferations.* There is abundant literature on potential ICC markers that could be used to separate benign from malignant mesothelial proliferations. Thus, while desmin is claimed to mark benign reactive mesothelium, epithelial membrane antigen (EMA, clone E29), p53, glucose transporter 1 (GLUT1), and insulin-like growth-factor 2 messenger ribonucleic acid (RNA)-binding protein 3 (IMP-3) are claimed to mark mesotheliomas.[52,53] However, there is too much overlap and variability in the expression patterns of these markers. Presently, two new biomarkers are being implemented to distinguish mesothelioma from reactive mesothelial cells: BRCA-associated protein 1 (BAP1) and p16/CDKN2A. Loss of nuclear BAP1 detected by ICC supports the diagnosis of mesothelioma, mainly of the epithelioid subtype (it is very uncommon in sarcomatous and desmoplastic variants).[52,53] The reported frequency of BAP1 protein loss in epithelioid mesotheliomas ranges from 55% to 80%.[52,54] If BAP1 is intact or a sarcomatoid mesothelioma is suspected, fluorescence *in situ* hybridization (FISH) for homozygous deletion of p16 should be tested.[54] Deletions of p16 are identified in up to 80% of pleural mesotheliomas (sarcomatoid subtype 90–100%, epithelioid and biphasic subtypes 70%). Neither BAP1 loss nor p16 deletions occur in reactive mesothelial cells.[54]

Mesothelioma versus *carcinoma.* Separation of mesothelioma from other malignancies is relatively straightforward using morphology and ICC stains. Virtually all epithelioid mesotheliomas and most sarcomatoid mesotheliomas exhibit immunoreactivity for pancytokeratin (i.e. multiple keratins including AE1/AE3, CAM 5.2, and CK5/6).[51] A combination of at least two positive markers of mesothelioma [e.g. calretinin, CK5/6, WT-1, and podoplanin (D2-40)] and two negative for the other tumor under consideration based on cytomorphology [e.g. EPCAM (also known as MOC31 or BerEP4), CEA, TTF-1 or napsin A, and claudin 4 in cases of possible carcinoma] is accepted for establishing a definitive diagnosis of the former.[47,51,55] A recent study of PF CBs from 258 patients with malignant PEs, of whom 53 had mesotheliomas, found BAP1 loss by ICC in 87% of mesotheliomas as compared with 2% in a variety of metastatic carcinomas that suggests a potential role of this marker not only in differentiating mesothelioma from reactive mesothelial proliferations as mentioned in the preceding text, but also from carcinoma.[56]

Pleural metastases of unknown primary site. Finally, in a carcinoma of unknown primary site involving the pleura, the use of appropriate ICC panels enables a precise diagnosis in many cases. It is common to start with the CK7/CK20 expression profile that roughly orientates toward a primitive origin of the neoplasm.[57] Subsequently, more tumor-specific markers are tested, such as TTF-1 and napsin A (nonsquamous lung cancer); GATA3, GCDFP15, mammaglobin and hormone receptors (breast); p40 and p63 (squamous-cell carcinomas); synaptophysin and chromogranin A (neuroendocrine tumors); CDX2 and cadherin 17 (gastrointestinal and pancreas); and PAX8 (ovary, kidney), among others.[58]

Molecular markers

Molecular markers are especially relevant in the context of malignant PEs due to lung cancer. The discovery of driver mutations and rearrangements for which targeted therapies are available have led to the recommendation of molecular testing as the standard approach to non-small cell lung cancer (NSCLC) patients. This should include, at minimum, testing for mutations in the genes encoding epidermal growth-factor receptor (EGFR) and BRAF (V600E), and for translocations in the genes encoding anaplastic lymphoma kinase (ALK) and rat osteosarcoma (ROS1) in patients with nonsquamous NSCLC.[59,60] In addition, the expression of programmed death ligand 1 (PD-L1) by ICC staining should be screened in patients with squamous-cell lung cancer and in those with nonsquamous NSCLC who do not have targetable mutations.[61] While subjects with targetable mutations should receive tyrosine kinase inhibitors, those with a PD-L1 status $>$ 50% are candidates for immune-checkpoint inhibitors.

Although tissue has been considered the standard material for molecular analyses, small

pleural biopsies and cytology fluid specimens are the only material available in almost 80% of cases.[62] Moreover, the concordance between the primary tumor and pleural metastases concerning mutational alterations is very high.[63] Therefore, PF samples (CBs, smears, cytospins or liquid-based cytology) are considered suitable alternatives to tissue biopsy for molecular studies, provided they are not paucicellular.[64,65] The adequacy of specimens for molecular testing should be determined by assessing cancer-cell content and deoxyribonucleic acid (DNA) quantity and quality. It is possible to enrich the tumor content of the sample by macro- or microdissection of a tumor area.[65]

ICC is the test of choice for ALK, ROS1 (in this case, positive results should be subsequently confirmed by FISH) and PD-L1 alterations, but it is not appropriate for EGFR and BRAF mutation testing. Instead, polymerase-chain-reaction-based or next-generation sequencing techniques are employed for EGFR mutations (including T790M mutations that confer resistance to EGFR-targeted kinase inhibitors), which can even be convincingly evaluated on circulating cell-free DNA from PF supernatants.[66] In the clinical setting where tissue or PF are limited/insufficient for molecular testing, the use of cell-free plasma DNA assays represents a good alternative.

The group of genes that must be offered to select NSCLC patients for targeted therapy is rapidly evolving. In a short time, additional genes such as MET, RET, ERBB2 and KRAS, will probably be used as part of multiplexed genetic sequencing panels. Specifically, the presence of a mutation in KRAS may identify patients who will not benefit from further molecular testing, owing to the low probability of overlapping targetable alterations.[60]

Future biomarkers

Ongoing research is directed at the discovery of novel biomarkers in blood, PF or pleural tissue for diagnostic purposes. There are studies involving the genomic (DNA), transcriptomic (all RNA types), proteomic (proteins) and metabolomic (small molecules or metabolites) exploration of a huge quantity of potentially unidentified biomarkers, using high-throughput methodologies. Unfortunately, they have not reached clinical implementation due to the inclusion of small sample populations, use of expensive technologies limited to research settings, and lack of a validation phase. Some illustrative examples follow.

At the post-transcriptional level, a number of candidate micro-RNAs (i.e. miR; small noncoding RNAs that contribute to regulating gene expression) in pleural tissue or body fluids have been tested as potential biomarkers of mesothelioma.[67] In this way, miR-126 has repeatedly been downregulated in plasma and formalin-fixed paraffin-embedded samples of mesothelioma patients. In a recent study, plasma levels of mi-R126 and mi-R132-3p were shown to be significantly lower in 22 patients with mesothelioma than in 44 asbestos-exposed controls.[68] The combination of these two micro-RNAs yielded 77% sensitivity and 86% specificity for labeling mesothelioma.

Concerning proteomics, a commercially available biochip array which contained 120 prespecified protein biomarkers related to cancer was tested on the PF samples of 105 patients in order to differentiate between malignant and TB effusions, as well as between lung adenocarcinoma and mesothelioma.[69] The selected candidate biomarkers were subsequently validated in an independent population of 102 patients, using more clinically applicable protein methodologies. A panel of four (metalloproteinase-9, cathepsin-B, C-reactive protein, chondroitin sulfate) and three (CA19-9, CA15-3, kallikrein-12) different protein biomarkers had highly discriminate properties for the preceding two comparisons (respective AUC of 0.98 and 0.94).[69] Currently, a prospective, multicenter study, named DIAPHRAGM (Diagnostic and Prognostic Biomarkers in the Rational Assessment of Mesothelioma), is trying to determine whether a multiplexed proteomic platform will provide clinically useful serum markers of mesothelioma.[70]

Lastly, the investigation of the metabolic profile of PE samples through a high-resolution proton nuclear magnetic resonance spectroscopy has yielded a limited number of metabolites (e.g. from lactic acid, amino acids or lipids)[71–73] which may potentially contribute to the distinction between benign and malignant etiologies.

Conclusion

PF biomarkers may provide such relevant information to clinicians that invasive diagnostic procedures, such as pleural biopsy (particularly *via*

Table 1. Diagnostic accuracy for pleural fluid/tissue tests that identify heart failure, tuberculosis and malignancy*.

	Sensitivity %	Specificity %	Comments
Heart failure			Diagnostic accuracy measures refer to the comparison between cardiac and noncardiac effusions (which also include other 'transudates'); NT-proBNP is the preferred test
Light's criteria	70–75	93–94	
Cholesterol < 45 mg/dl	85–90	70–75	
S–PF albumin gradient > 1.2 g/dl	90–95	60	
S–PF protein gradient > 3.1 g/dl	80	80	
S–PF protein gradient > 2.5 g/dl	90–95	65	
NT-proBNP > 1500 pg/ml	94	91–94	
Tuberculosis			ADA is the most widely used test for tuberculosis diagnosis in areas with moderate-to-high prevalence of the disease; in low-prevalence areas, ADA can be used as a rule-out test
Closed pleural biopsy	80	95	
Thoracoscopic biopsy	99	95	
PF culture	31	100	
ADA > 35–40 U/l	93	92	
Interferon-γ	89	97	
Interleukin-27	94	92	
Malignancy			Some of these test yields depend greatly on the tumor type (i.e. lower sensitivities are expected in mesotheliomas, squamous-cell carcinomas and sarcomas)
Closed pleural biopsy	48	100	
CT-guided pleural biopsy	85	100	
Thoracoscopic pleural biopsy	93–95	100	
PF cytology	45–60	100	
CEA > 45 ng/ml	41	100	
CA 15-3 > 77 IU/l	40	100	
PF mesothelin (various cutoffs)	79	85	
Loss of BAP-1 by ICC$	55–85	98–100	
p16/CDKN2A deletions by FISH‡	70–90	100	
EGFR mutations§	65–80	80–95	

*Information from references 4, 10, 12, 25, 28, 31, 38, 40, 44, 52 and 53.
$For labeling mesothelioma.
‡For distinguishing malignant from benign mesothelial proliferations.
§For non-small cell lung cancer.
ADA, adenosine deaminase; CEA, carcinoembryonic antigen; CT, computed tomography; EGFR, epidermal growth-factor receptor; FISH, fluorescence *in situ* hybridization; ICC, immunocytochemistry; NT-proBNP, amino-terminal fragment of probrain natriuretic peptide; PF, pleural fluid; S–PF, serum minus pleural fluid levels.

thoracoscopy), may be unnecessary.[37] There is firm evidence to support the routine use of NT-proBNP and ADA for the diagnosis of HF and TB, respectively. There is enough evidence for testing the loss of BAP1 by ICC and p16 deletion by FISH methodologies when differentiation between mesothelioma and reactive benign mesothelial cells is a concern, though these markers are not yet widely employed. Generally, there is uniformity in the markers that should be included in ICC panels for separating mesothelioma from adenocarcinoma. Lastly, molecular testing on PF specimens is becoming a feasible alternative to tissue biopsy for phenotyping malignant PEs in lung cancer patients. Some common biomarkers and ancillary techniques for testing PEs are summarized in Table 1.

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Conflict of interest statement

The author declares that there is no conflict of interest.

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